Reduced intracortical inhibition and facilitation in the primary motor tongue representation of adults who stutter

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**Abstract**

Objective: We aimed at detecting neurophysiological changes, in the primary motor tongue representation in adults with persistent stuttering.

Methods: Using transcranial magnetic stimulation in 12 patients and 14 controls, we examined motor threshold, motor-evoked potential (MEP) input–output curve, short-term intracortical inhibition (SICI) and intracortical facilitation (ICF), based on eight trials per conditioning-test interval.

Results: In controls inhibition of the MEP-amplitude at short inter-stimulus intervals (ISI) and facilitation of the MEP-amplitude at long ISIs was evident. Patients showed an inhibition at ISI 3 ms and weaker non-significant inhibition at ISI 2 ms; this delay of inhibitory activity was especially prominent in the right hemisphere. Facilitation was reduced at ISI 10 and 15 ms in patients. Furthermore, MEP input–output curve was steeper in patients. Motor thresholds did not differ between groups.

Conclusions: In persistent stuttering intracortical excitability of the primary motor tongue representation is altered with a deviant time course for inhibitory activity in the right hemisphere and reduced paired-pulse facilitation.

Significance: These results specify changes in intracortical networks possibly mediated by altered GABAergic regulations in persistent stuttering. Thus, a better understanding of pathomechanisms and a potential role in understanding pharmacological treatment responses emerge by using transcranial magnetic stimulation.

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1. Introduction

Speech-relevant cortical and subcortical neural systems appear to be malfunctioning in persistent stuttering (Fox et al., 1996; Ludlow and Loucks, 2003; Brown et al., 2005). This speech disorder affects approximately 1% of the adult population, severely compromising their quality of life (Craig et al., 2002; Bloodstein and Ratner, 2008). Stuttering is characterized by involuntary, intermittent interruptions of fluent speech. Speech sound and syllable repetitions, sound prolongations and speech blocks are prominent signs. It is still unclear which changes in motor cortical function contribute to these dysruptions to a smooth execution of complex spatiotemporal commands of articulatory gestures.

Current knowledge about cortical mechanisms in stuttering relies mainly on imaging studies reporting for instance a right-hemispheric hyperactivity in the primary motor cortex during dysfluent speech (Fox et al., 1996, 2000; Braun et al., 1997). This hyperactivity has been speculated to be related to an increased cortical excitability (Ludlow and Loucks, 2003). In physiological terms motor cortical excitability can be explored using transcranial magnetic stimulation (TMS). TMS-studies in stuttering are scarce, although this technique is an established tool to assess noninvasively the functional integrity of descending corticospinal and corticonuclear pathways of the human motor cortex. Suprathreshold TMS pulses create a series of action potential volleys originating from layer 5 pyramidal cells in the primary motor cortex. Conducted along the descending motor-pathways this activity can be registered in epidural recordings as a sequence of a direct (D) wave, a first indirect, monosynaptically evoked 11-wave and later indirect waves I2 and I3 (Ziemann and Rothwell, 2000). The ultimate effect of the suprathreshold TMS stimuli is a brief response in the target muscle, quantified by the motor-evoked potential (MEP). Subthreshold TMS stimuli can activate infracortical circuits in the motor cortex (Paulus et al., 2008). Paired-pulse stimulation with a subthreshold conditioning stimulus (CS) followed by a suprathreshold test stimulus (TS) induces either a reduced motor cortex excitability, short-term intracortical inhibition (SICI); or an increased motor cortex excitability, the intracortical facilitation (ICF). SICI and ICF depend on the length of the inter-stimulus interval (ISI) between CS and TS (Kujirai...
et al., 1993; Ziemann et al., 1998). SICI is mainly brought about by attenuation of later action potential volleys, while D-wave and I wave are largely unaffected (e.g., Hanajima et al., 1998). Fig. 1 depicts modulated intracortical excitability in a typical healthy subject. Hence, TMS is a tool for transsynaptically activating cortical neurons, providing a method to assess the strength of intracortical synaptic connections.

Neurons of the primary motor cortex (M1) receive influential input from frontal cortex regions and from the basal ganglia; their descending output innervates coordinated, voluntary movements. Intracortical inhibitory and facilitatory networks within M1 may promote the selection and initiation of target movements and suppress synergisms. Speech requires a highly coordinated interplay between different subsystems innervated bilaterally by six different cranial nerves (V, VII, IX, X, XI, XII). Respiratory activity has to be synchronized as well, again involving paired spinal nerves. The excitability of the cortical neurons that project through corticobulbar pathways to the target cranial nerves and further to the target muscles is shaped by subcortical and intercortical as well as intracortical circuitry. Intracortical excitability modulation is induced by interactions of inhibitory and excitatory circuits.

A disturbed intracortical excitability of neurons in the primary motor cortex (M1) that project to speech relevant muscles is a potential reason for the dysfluent speech (Ludlow and Loucks, 2003), but the intracortical excitability of speech relevant motor cortex regions had not been investigated in adults who stutter (AWS). An adjacent region in M1, the hand representation, was studied in our group (Sommer et al., 2003) with the finding of an unaltered intracortical excitability in AWS.

Altered intracortical inhibition in stuttering might be a reasonable expectation because imbalanced cortical excitability in motor regions has been implicated by functional neuroimaging during symptom production (Brown et al., 2005). Besides, stuttering shares clinical features of other movement disorders: tic-like involuntary movements (Mulligan et al., 2003), focal dystonia-like excessive activation of task-related and task-unrelated muscles (Somm er et al., 2003) and Parkinsonism-like freezing of articulatory gestures (Alm, 2008). All these movement disorders are characterized by a reduced SICI (Berardelli et al., 2008).

In the present study, we used TMS elicited motor evoked potentials (MEPs) to test the hypothesis that intracortical excitability in the M1 tongue representation is altered in adults who stutter (AWS). Specifically, we hypothesized that the excitability of inhibitory circuits within M1 tongue representation is reduced in AWS. A decreased intracortical inhibition may lead to a reduced inhibition for the prevention of movements (Stinear et al., 2009) thereby contributing to the intermittent dysfluencies in stuttering.

2. Methods

2.1. Subjects

Twelve AWS (three female; mean 29.9 years, SD 8.2) and 14 fluent speakers (FS, 3 females, mean 29.5 years, SD 7.6) participated in this study. Adults who stutter were recruited from the local stuttering support group and the Institute for the Kassel Stuttering Therapy (Euler et al., 2009). Fluent speakers were recruited by advertisement. The groups were matched for age, handedness (Oldfield, 1971) and education. Seven AWS reported a family history of stuttering. None of the FS reported having a family history of speech or language disorders. Expect from stuttering in the AWS group, participants reported no medical history, neurological impairment or drug use that would potentially affect their neurological function. Before experimental measures were obtained with TMS, all subjects were screened for exclusion criteria using a standard TMS safety screen (Keel et al., 2001). All subjects provided written informed consent prior to inclusion into the study. This study received ethical approval from the Goettingen Ethics Committee.

2.2. Fluency assessment

To judge stuttering severity, fluency assessments were performed regarding the German version of the SSI-3 (Sandriesser and Schneider, 2008). Speech samples of all participants containing a conversation about job or school and a reading task were videotaped and analyzed by a qualified speech–language pathologist. SSI-3 norms were adapted from Riley (1994). The offline analysis of dysfluencies included 500 syllables for the conversation and not less than 340 syllables for the reading task. Sound prolongations, blocks (silent prolongation of an articulatory posture) as well as sound and syllable repetitions were counted as stuttered syllables. Monosyllabic words that were repeated with apparent undue stress or tension were counted. Furthermore, the estimated duration of the three longest blocks and observation of physical concomitants were included for the estimate of stuttering severity in AWS.

2.3. Experimental procedures

Subjects were seated in a comfortable chair. Bilateral simultaneous surface recordings of the lingual muscle were taken with two pairs of disposable pre-gelled silver/silver chloride ring electrodes (5 mm × 100 mm, ViaSys Neurocare, Hoechberg, Germany).
Electrodes were mounted at a customized spoon-shaped silicon mouthpiece. Contact area at the tongue was 5 mm x 10 mm at longitudinal and lateral inter-electrode distances of 25 and 20 mm, respectively. The mouthpiece was placed on the upper surface of the tongue, and the subjects were asked to close their lips and teeth leisurely without additional pressure and to hold the end of the mouthpiece with the hand ipsilateral to TMS stimulation site, with the elbow comfortably supported. While recording participants were asked to push the tongue tightly against the electrodes and their lower teeth. This procedure was adapted from Rödel et al. (2003).

Surface EMG signals were recorded using a CED power 1401 interface with a sampling frequency of 5 kHz, amplified 1000X and Butterworth bandpass filtered between 20 and 2000 Hz. Recordings were controlled by Signal Software (Cambridge Electronic Design, version 2.13).

2.4. Transcranial magnetic stimulation

**Experiment 1**

TMS was applied while participants sat comfortably in a reclining chair. Subjects were instructed to stay relaxed throughout the assessment. The voluntary background contraction of the tongue was maintained at approximately 10% of maximum activity (Muellbacher et al., 2001). Muscle activation was controlled through visual feedback of EMG activity. TMS was achieved using a monophasic stimulus applied through a figure-of-eight coil with an outer wing diameter of 70 mm. The coil was positioned tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. In this position, the induced current flow in the brain was in the posterior-anterior direction. The scalp surface was explored systematically and the position for consistently inducing maximal MEPs in the contralateral tongue site at the lowest stimulus strength was identified as the “hot spot” and marked with a pen to ensure accurate coil placement throughout the experiment (Muellbacher et al., 2001). We found the motor tongue representation to be slightly more anterior and more lateral than what we usually observe for the hand representation which is consistent with the literature (Svensson et al., 2003). Motor threshold for SICI and ICF was assessed with the coil connected to a Bistim module (Magstim Company Ltd., Whitland, Wales), which connected two identical Magstim200 stimulators. Single TMS pulses were applied to determine the minimal stimulus intensity to the nearest 1% of the maximum stimulator output required to produce MEPs of greater than 100 μV in at least three of six consecutive stimuli. This intensity defines the motor threshold (MT). Intracortical excitability was assessed according to a paired conditioning test stimulus paradigm (Kujirai et al., 1993; Muellbacher et al., 2001), with a subthreshold conditioning stimulus followed by a supra-threshold test stimulus at different inter-stimulus intervals (ISIs). Four ISIs, 2, 3, 10 and 15 ms were tested and randomly intermixed with the test stimulus given alone. Each of the four ISIs was applied eight times while the test stimulus alone was applied 18 times. The conditioning stimulus was applied at 90% MT and the test stimulus was set at 130% MT. These ISIs and stimulation intensities were chosen based on findings of a previous study to obtain sizeable SICI and ICF in lingual muscles (Muellbacher et al., 2001).

This protocol was conducted in 14 FS and in 12 AWS in both hemispheres in a pseudo-randomized order in two separate sessions.

**Experiment 2**

Motor threshold was reassessed and MEP input–output curves were determined with the same coil, positioned as described before, but this time connected to a single pulse Magstim200 stimulator (Magstim Company Ltd., Whitland, Wales). The MEP input–output curve was recorded using six intensity levels between 90% and 140% of MT (10% increments). Five stimuli were applied at each intensity level in a consecutive order every 4 s. Afterward the MEP input–output curve was recorded while participants were asked to contract their tongue with about 60% maximum activity with a short (1 min) break between intensity levels. This protocol was conducted in both hemispheres in a pseudo-randomized order in two separate sessions, in 12 FS and 8 AWS who also participated in Experiment 1.

2.5. Data analysis

Altogether participant recruitment comprised 17 AWS and 17 FS. We did not conduct the whole procedure in four AWS and in three FS because M1 tongue representation could not be determined properly in one or both hemispheres of these subjects with TMS intensities weak enough to allow proper intensities required to proceed with the paired-pulse protocol. One other AWS was excluded because fluency assessment yielded an additional cluttering component in this patient. According to the definition (WHO, 2007) cluttering was recognized by rapid, erratic and dysrhythmic speech dysfluency with distinct speech timing abnormalities.

To determine pre-TMS tongue activity we analyzed the EMG signal of all valid recordings of every single subject. Recordings with TMS artifacts outlasting the motor evoked response were excluded. For the paired-pulse protocol 60 ms of the EMG signal immediately before the TMS artifact and for the input–output curve data 79 ms of the EMG signal were considered. These signals were corrected for offset, rectified and averaged using Matlab.

Motor evoked potential peak-to-peak amplitudes were analyzed with Signal 4.04 (Cambridge Electronic Design). Mean peak-to-peak amplitudes were calculated for each condition, including unconditioned MEP amplitudes of the MEP input–output curve procedure and of the paired-pulse procedure, and conditioned MEP amplitudes of the paired-pulse procedure for ISI 2, 3, 10 and 15 ms for either projection and either hemisphere. The conditioned MEP amplitudes were normalized and are given as ratios of the unconditioned MEP amplitude recorded in the paired-pulse protocol.

In addition to the peak-to-peak amplitude, MEP magnitude was also estimated by the area under the baseline corrected and rectified EMG signal, where baseline was defined as average pre-TMS amplitude of the EMG. The time interval of significant MEP response was defined manually for each trial and each recording site. The interval selection was guided by the overall shape of the MEP amplitude envelope and by the rate of change of MEP amplitude. Selection of intervals and computation of the area under the curve was performed in a custom written EMG-Browser in Igor Pro (Wavemetrics).

2.6. Statistical analysis

To test for group differences for the variables age and percentage of stuttered syllables we used two-tailed t-tests for independent samples; for the t of percentage of stuttered syllables heterogeneity of variance was stated; education and handedness were analyzed with Mann–Whitney U-tests. Nonparametric testing was chosen since education is an ordinarily scaled variable (1 = school, 2 = high school, 3 = less than 2 years college, 4 = 2 years college, 5 = 4 years college, 6 = postgraduate) and handedness did not show normal distribution in either group.

**Experiment 1**

Motor threshold comparison was calculated with repeated-measures ANOVA with hemisphere (left, right) as a within-subjects factor and group (AWS, FS) as a between-subjects factor.
Unconditioned MEP amplitudes were tested by $2 \times 2 \times 2$ repeated-measures ANOVA with the between-subjects factor group (AWS, FS) and the within-subjects factors hemisphere (left, right) and projection (contralateral, ipsilateral).

For SICI and ICF analyses, conditioned MEP amplitudes were expressed as a percentage of unconditioned MEP amplitudes (test stimulus only condition), employing a $2 \times 2 \times 2 \times 2 \times 4$ omnibus ANOVA with the between-subjects factor group (AWS, FS) and the within-subjects factors hemisphere (left, right), projection (contralateral, ipsilateral) and ISI (2, 3, 10, 15 ms).

Separate $2 \times 2 \times 2 \times 2$ repeated-measures ANOVAs were performed on SICI and ICF data with the within-subjects factors hemisphere (left, right), projection (contralateral, ipsilateral) and ISI (1, 2, 3, 10, 15 ms) and the between-subjects factor group (AWS, FS).

To test potential correlations between stuttering severity and intracortical excitability, we calculated Spearman's correlation coefficients between the stutterer's SSI-3 overall-scores and the normalized values of ICI, ICF and MT, respectively. The two-tailed significance level has been considered.

Pre-TMS tongue activity and unconditioned MEP amplitude were assessed separately using $2 \times 2 \times 2$ repeated-measures ANOVAs with within-subjects factors hemisphere (left, right) and tongue site (left, right) and between-subjects factor group (AWS, FS).

To test potential correlations between stuttering severity and intracortical excitability, we calculated Spearman's correlation coefficients between the stutterer's SSI-3 overall-scores and the normalized values of ICI, ICF and MT, respectively. The two-tailed significance level has been considered.

### 3. Results

#### 3.1. Experiment 1

**3.1.1. Participants**

The groups matched for age, $t(24) = -0.13, p = 0.9$ (unpaired two-tailed $t$-test), handedness, $p = 0.980$ (U-test) and education, $p = 0.206$ (U-test). Adults who stutter produced more stuttered syllables than fluent speakers, $t(11.054) = -4.96; p < 0.001$ (unpaired two-tailed $t$-test, heterogeneity of variance). Stuttering severity was very mild in five, mild in two, moderate in two, severe in two and very severe in one AWS. Averaged stuttering onset was at age $4.5 \pm 2.8$ (see Table 1 for demographics and fluency scores).

**3.1.2. Motor threshold**

The values of MT are given in Table 1. Analysis of variance considering the factors group and hemisphere yielded no effects or interaction. Previous TMS studies of the primary motor hand area in AWS resulted in contradictory observations reporting increased motor thresholds in AWS relative to FS (Sommers et al., 2003), as well as no differences (Sommers et al., 2009; Neef et al., in press).

**3.1.3. Pre-TMS tongue activity**

Pre-TMS tongue activity in the paired-pulse protocol was similar in both groups (Fig. 2A). ANOVA yielded no effect for hemisphere, projection and group as well as no interactions.

**3.1.4. Unconditioned MEP amplitude**

A single unconditioned TMS pulse with 130% MT results in similar MEP amplitudes for FS and AWS, ANOVA detected no effect of group. MEP amplitudes at contralateral projections were significantly larger than at ipsilateral projections (effect of projection $F(1, 24) = 15.44, p = 0.001$). Post hoc group-wise comparisons of contralateral and ipsilateral MEP amplitudes via two-tailed, paired $t$-tests yielded significant differences for all conditions, expect the right hemisphere projections in FS (see Table 2). This finding is in accordance with previous studies and likely reflects a predominance of the contralateral projections present even in the tongue (Meyer et al., 1997; Rödel et al., 2003). Analysis of variance yielded no other effects.

**3.1.5. Intracortical excitability**

In FS ISIs of 2 and 3 ms lead to inhibition of the MEP amplitude, whereas ISIs of 10 and 15 ms lead to facilitated the MEP amplitude (Fig. 2B). Post hoc paired $t$-tests revealed significant changes in intracortical excitability for all ISIs in FS with a significance level consistently smaller than 0.0001. These results agree in time with previous studies and likely reflects a predominance of the contralateral projections present even in the tongue (Meyer et al., 1997; Rödel et al., 2003). Analysis of variance yielded no other effects.

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### Table 1

Description of the samples. Given are the means ± standard deviations; minimum–maximum. Stuttering onset and age were documented in years; stuttering severity was estimated with the Stuttering Severity Index (Sandrieser and Schneider, 2008); stuttered syllables were mean percentage out of not less than 340 read and 500 spoken syllables; handedness index was calculated (Oldfield, 1971); education was classified as follows: 1 = school, 2 = high school, 3 = less than 2 years college, 4 = 2 years college, 5 = 4 years college, 6 = postgraduate; motor threshold (MT) was quantified in percent of maximum stimulator output in the left and right hemisphere, MT_2 threshold determine with the Bistim module, and MT_1 threshold determined with a single Magstim200 stimulator. Note that MT_2 yields higher values than MT_1 because of the power loss due to the bistimulation module.

<table>
<thead>
<tr>
<th>Adults who stutter</th>
<th>Fluent speakers (N = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1 (N = 12)</strong></td>
<td><strong>Experiment 2 (N = 8)</strong></td>
</tr>
<tr>
<td><strong>Stuttering onset</strong></td>
<td><strong>Stuttering severity</strong></td>
</tr>
<tr>
<td>4.5 ± 2.8; 2.0–10.0</td>
<td>23.3 ± 9.3; 10–39</td>
</tr>
<tr>
<td>5.1 ± 3.3; 2–10</td>
<td>22.8 ± 10.2; 10–39</td>
</tr>
<tr>
<td>5.1 ± 2.2</td>
<td>11.054</td>
</tr>
</tbody>
</table>

course and magnitude with a previous report (Muellbacher et al., 2001). In AWS excitability was significantly changed for ISI 3 ms, $t(11) = 4.9, p < 0.0001$; 10 ms $t(11) = 3.75, p = 0.003$; and 15 ms $t(11) = 2.4, p = 0.035$; but inhibition at ISI 2 ms did not reach significance, $t(11) = -1.5, p = 0.15$. Fig. 2B depicts the main effect of ISI with $F(1, 24) = 87.72, p < 0.0001$ Thus, short ISIs at 2 and 3 ms significantly inhibited the MEP amplitude in both groups expect for ISI 2 ms in AWS, while longer ISIs at 10 and 15 ms significantly augmented motor evoked responses. The interaction between ISI and group with $F(3, 22) = 7.69, p < 0.0001$ indicates that the magnitude of the effect differs between the two groups.

### SICI

Short-term intracortical inhibition was reduced for the right hemispheric projections at an ISI 2 ms in AWS compared to FS (Fig. 2D and F). ANOVA yielded an effect of projection $F(1, 24) = 4.36, p = 0.048$; an effect of ISI $F(1, 24) = 9.34, p = 0.005$; an interaction of ISI and group $F(1, 24) = 4.59, p = 0.043$; and an interaction of hemisphere, ISI and group $F(1, 24) = 5.41, p = 0.027$.

Post hoc $t$-tests revealed a significantly reduced SICI of the ipsilateral projections of the right hemisphere at an ISI of 2 ms in AWS $t(24) = -2.1, p = 0.046$; for the same hemisphere and ISI, SICI trends to decreased inhibition for the contralateral projection $t(24) = -2.0, p = 0.056$.

Analysis of variance yielded no other effects. Additionally we calculated separate $2 \times 2$ ANOVAs for each hemisphere and its contralateral projecting and show the results in Table 3.

### ICF

Intracortical facilitation was consistently reduced in AWS (Fig. 2C–F). ANOVA yielded an effect of group $F(1, 24) = 10.34, p = 0.004$; and an interaction of hemisphere and projection $F(1, 24) = 6.06, p = 0.021$. 

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Post hoc unpaired, two-tailed t-tests revealed significantly reduced ICFs for right hemispheric contralateral projection at either ISI (10 ms, \(p = 0.005\); 15 ms, \(p = 0.011\)) and for the ipsilateral projection at ISI 15 ms (\(p = 0.024\)). Left hemispheric contralateral projections exhibited a significantly reduced ICF at ISI 10 ms (\(p = 0.034\)). Table 4 contains \(p\) values for all conditions documenting a reduced ICF for all conditions at a level of marginally significance (\(p < 0.1\)) in AWS related to FS. Analysis of variance yielded no other effects. Additionally we calculated separate 2 × 2 ANOVAs for each hemisphere and its contralateral projecting and show the results in Table 3.

3.1.8. Correlation analysis

Correlation analyses yielded no correlation between stuttering severity and ICI, ICF and MT, respectively.

3.2. Experiment 2

3.2.1. Participants

The groups matched for age, \(t(20) = -0.24, p = 0.811\) (unpaired two tailed t-test), handedness, \(p = 0.680\) (U-test) and education, \(p = 0.205\) (U-test). Adults who stutter produced more stuttered syllables than fluent speakers, \(t(7.02) = -3.91; p = 0.006\) (unpaired two tailed t-test, heterogeneity of variance). Stuttering severity was very mild in three, mild in two, moderate in one, severe in one and very severe in one AWS. Averaged stuttering onset was at age 5.1 ± 3.3 (see Table 1 for demographics and fluency scores).

3.2.2. Motor threshold

In FS MT was for the left and right hemisphere 42.6 ± 6.7% and 44.3 ± 8.8% maximum stimulator output, respectively. AWS had a MT for the left and right hemisphere of 42.8 ± 8.4% and 40.8 ± 5.9% maximum stimulator output. ANOVA yielded no significant effects or interaction (see Table 1 and Fig. 3B).

3.2.3. Pre-TMS tongue activity

Pre-TMS tongue activity in the MEP input–output curve data did not differ between groups (Fig. 3A). ANOVA yielded a significant effect of mode \(F(1, 18) = 9.33; p = 0.007\), with an increased tongue activation at a contraction of approximately 60% of maximum contraction 0.048 ± 0.05 mV versus tongue activation at a contraction of approximately 10% of maximum contraction 0.014 ± 0.01 mV (see Fig. 3A).

3.2.4. MEP input–output curve

The ANOVA considering MEP peak-to-peak amplitude revealed an effect of mode, \(F(5, 14) = 11.45, p < 0.001\) which reflects that MEP amplitudes are larger while the tongue is more strongly contracted (mean MEP 1.01 ± 0.67 mV) compared to less contraction (mean MEP 0.55 ± 0.28 mV). Furthermore, analysis yielded an effect of projection, \(F(1, 18) = 31.29, p < 0.001\) with larger MEP amplitudes in the contralateral projection (0.91 ± 0.48 mV) than in the ipsilateral projection (0.65 ± 0.38 mV). This again reflects slightly stronger contralateral projections and is in accordance with previous reports from other labs (Muellbacher et al., 2001). The effect of stimulus intensity, \(F(5, 14) = 16.49, p < 0.001\), is reflected in the steady increase of MEP amplitudes with increasing stimulus intensity (90%, 0.31 ± 0.19 mV; 100%, 0.44 ± 0.27 mV; 110%, 0.65 ± 0.35 mV; 120%, 0.91 ± 0.55 mV; 130%, 1.1 ± 0.68 mV; 140%, 1.28 ± 0.62 mV; see Fig. 3C). Analysis yielded an interaction between stimulus intensity and group, \(F(5, 14) = 2.74, p = 0.023\), because MEP recruitment was steeper in AWS related to FS (Fig. 3B). Post hoc unpaired t-tests of MEP amplitudes yielded no significant differences between groups for separate conditions. Additionally, we found an interaction between mode and stimulus intensity, \(F(1, 18) = 3.86, p = 0.003\), because the slope of the recruitment curve under 10% contraction was steeper at lower intensities and flatter at higher intensities compared to the recruitment curve under 60% contraction which showed the reversed pattern (Fig. 3E). Finally, there was an effect of mode, hemisphere and projection, \(F(5, 14) = 6.23, p = 0.02\), because under 60% contraction motor responses were enlarged in the contralateral projection of the left hemisphere (1.29 ± 1.06 mV) compared to the contralateral projection of the right hemisphere (1.05 ± 0.66 mV).

The ANOVA considering MEP area yielded one additional interaction between projection and stimulus intensity with
Areas of the MEP of the contralateral projection had a steeper slope compared to the MEP areas of the ipsilateral projection (Fig. 3F).

Furthermore, the ANOVA with MEP areas yielded almost the same effects compared to the ANOVA with MEP peak-to-peak amplitudes: effect of mode $F(1, 18) = 10.95; p = 0.004$; effect of projection $F(1, 18) = 34.12, p < 0.0001$, effect of stimulus intensity $F(5, 14) = 30.9; p < 0.0001$; an interaction of mode, hemisphere and projection $F(5, 14) = 4.92, p = 0.04$, and an interaction between mode, stimulus intensity and group $F(5, 14) = 2.38; p = 0.045$. Post hoc repeated measures ANOVAS separated for mode yielded in an significant interaction between group and intensity at 60% of maximum contraction, $F(5, 14) = 2.55; p = 0.033$. This interaction was missing in the 10% of maximum contraction mode. Post hoc unpaired $t$-tests yielded no differences between groups concerning the MEP area at 60% maximum contraction at 140% MT or at 130% MT although the slope is steeper in AWS in this condition (Fig. 3D).

### 4. Discussion

Here we present the first assessment of intracortical excitability in the M1 representations of the lingual muscle in stuttering. The reduction of short-term intracortical inhibition at ISI 2 ms in our sample of stuttering subjects partly confirms our hypothesis of a
reduced ICI. Unexpected were the observations of a generally reduced intracortical facilitation, and of a steeper MEP recruitment in adults who stutter relative to fluent speakers.

4.1. Reduction of short-term intracortical inhibition in stuttering

The reduction of SICI indicates an altered excitability modulation of intracortical neural networks in stuttering. Due to the local action of TMS and the limited conduction velocities and synaptic delays it is assumed that SICI is mediated by the local neuronal circuits in the motor cortex (Di Lazzaro et al., 1998; Ziemann and Rothwell, 2000). At intervals larger than 1 ms SICI is caused by the activation of GABAergic interneurons and the subsequent inhibition of excitatory neurons (Fisher et al., 2002; Hanajima et al., 2003). We speculate that in AWS’s M1 tongue representation the interneuronal inhibitory network is less active at early times. In the next paragraph we shortly introduce the cellular physiology framework to discuss the effect.

A TMS pulse stimulates nerve fibers most likely at their terminations (Maccabee et al., 1993; Rotem and Moses, 2008) thereby activating synaptic terminals in all cortical layers. A suprathreshold TMS pulse activates enough excitatory synapses to elicit action potentials in layer 5 excitatory cells, the cortical output responsible for muscle activation. The layer 2/3 excitatory cells are activated as well, and in turn stimulate layer 5 cells, thereby prolonging and increasing the motor output. If the TMS pulse is weaker, excitatory cells are not sufficiently activated to fire action potentials, no motor response is elicited; the TMS stimulus is called subthreshold. Even in this case, however, the inhibitory interneurons are depolarized enough to fire action potentials. Those action potentials reach inhibitory (GABAergic) synapses onto the layer 2/3 and layer 5 excitatory neurons after a distance dependent conduction delay of 0–1 ms (Esser et al., 2005). Over the next 2 ms the inhibitory postsynaptic currents in the excitatory neurons increase. If a second, stronger TMS pulse is applied during this period, it might still suffice to activate the excitatory layer 5 neurons, that cause the motor response but due to the built up inhibition, the excitation of layer 5 and especially layer 2/3 neurons is weaker, leading to a reduction of action potential number, when compared to a single, unconditioned TMS stimulus. This view is supported by large scale modeling (Esser et al., 2005).

Our findings indicate that at an ISI of 2 ms for FS there is significant inhibition but not in AWS. In the right hemisphere in AWS the inhibition takes longer to develop. This delay of the peak inhibitory activity implies that inhibitory inputs on the excitatory cells develop with a slower time course in AWS. The reasons can be diverse, including altered kinetics of synaptic signaling (altered subunit composition of GABA receptor complexes), longer conduction delays (a larger fraction of long range, i.e., 1 mm, inhibitory connections (Kang et al., 1994) or short term synaptic plasticity, e.g., synaptic depression due to depletion of release-competent synaptic vesicles at the excitatory synapses innervating the layer 5 excitatory neurons.

GABAergic interneurons seem to play a key role in mediating the effect of intracortical inhibition. Therefore it is interesting to note that stuttering can be induced by theophylline (Movsessian, 2005) an adenosine receptor antagonist which has been previously described to reduce the binding of GABA to GABA receptors via a decoupling of the benzodiazepine binding site that is present on the receptor (Roca et al., 1990). Additionally, theophylline plasma concentration correlates positively with a reduction of SICI in healthy subjects (Nardone et al., 2004). SICI is mediated by either the α2- or α3-subunit of the GABA A receptor (Di Lazzaro et al., 2006). Thus, the susceptibility of the speech motor system in stuttering might be mediated by GABA A neurons.

An alternative explanation is that the reduction in SICI is caused by disproportionately strong contribution of those insensitive early waves to the MEP in AWS, because the subthreshold prepulse affects the late l-waves much stronger than the D-wave or the I1-wave. Studies in the hand representation demonstrated that a change in TMS-induced current direction can reveal the differential modulation of the individual waves in SICI (Hanajima and Ugawa, 2008); whether this is also the case for the bilateral tongue representation remains to be studied.

In addition, the missing correlation of reduced SICI for an ISI of 2 ms in the right hemisphere in AWS and stuttering severity does not suggest a major role of SICI in explaining the pathophysiology of stuttering. This could be related to the task-dependent nature of the disease.

4.2. Reduced intracortical facilitation in stuttering

One possible explanation for a generally reduced ICF in stuttering might be a different level of pre-TMS tongue activation. It is known that orofacial muscles are rarely at rest (Devlin and Watkins, 2008) and that muscle activation reduces ICF suggesting that voluntary drive reduces the excitability of intracortical circuits (Ridding et al., 1995). Therefore one might conclude that the pre-TMS tongue activity was higher in AWS and hence ICF was reduced. However, the comparison of pre-TMS tongue activity revealed no group differences. Consistent with this, previous EMG studies in stuttering do not provide evidence for elevated tonic activity or a co-activation in the laryngeal or orofacial muscles, neither during fluent speech nor during dysfluent speech (Smith et al., 1993, 1996). Even at rest lower lip activity did not differ between AWS and FS, while upper lip activity has been reported to be lower in AWS (de Felicio et al., 2007).

Another possible explanation for a reduced ICF might be that the MEP amplitude was already saturated in AWS at a test pulse intensity of 130% MT. This point also does not hold true because the MEP recruitment until 140% MT resulted in a steeper slope in AWS and the comparison of MEP areas between groups yielded no differences. Thus, our findings implicate a diminished excitability of excitatory neuronal circuits in motor cortex in the sample of AWS examined in the current study.

While ICF is normal in the M1 hand representation in AWS (Sommer et al., 2003) the current data imply a reduced intracortical facilitation for the M1 tongue representation. In contrast to ICI which is reduced in several movement disorders, reduced ICF has been described only in few movement disorders; unequivocally in cerebellar ataxia and equivocally in Huntington’s disease (Berardelli et al., 2008). The fact, that these movement disorders as well as movement disorders that are characterized by reduced ICI are related to a dysfunction of the basal ganglia or the cerebellum, suggests that the dysfunction in these structures might influence intracortical circuits that modulate motor cortex excitability. In stuttering the contribution of altered cerebello-cortical loops (Lu et al., 2010) as well as deviant basal ganglia function are proposed (Alm, 2004). Clinical trials with dopamine antagonists resulted in a positive effect on speech fluency (Burns et al., 1978; Maguire et al., 2010) while dopamine agonists enhanced dysfluency. An early study with Positron Emission Tomography reports an increased uptake of presynaptic dopamine in stuttering which indicates that stuttering is related to a hyper-dopaminergic status (Wu et al., 1997). Although, physiological mechanisms of ICF are not well understood we here speculate that the reduced ICF might be mediated by disturbed interaction between cortical and subcortical networks modulating inhibitory and facilitatory intracortical circuits.

Although, TMS paired-pulse techniques employed to study SICI and ICF in different movement disorders provide important
information about the pathophysiologies in the primary motor cortex (Hanajima et al., 2008), the exact mechanisms of these modulations of cortical excitability are still a matter of debate (Reis et al., 2008). Furthermore, literature on intracortical excitability in speech relevant muscles is scarce. Because the tongue muscles are rarely at rest and the innervations occurs bilaterally a direct comparison with previous literature on SICI and ICF is limited and restricts general conclusions.

4.3. MEP recruitment

The recording of the MEP input–output curve was necessary to address the question, whether MEP amplitude was already saturated in AWS in the facilitatory paired-pulse condition. To take care for a potential ceiling effect we obtained MEP input–output curves under different conditions of tongue activity. While recruitment under 10% of maximum tongue contraction was characterized by a steep slope at high intensities, MEP recruitment seems to reach saturation at high intensities under 60% contraction (Fig. 3E). Fig. 3C and D detail that this saturation is more clearly displayed in FS compared to AWS were recruitment between 130% and 140% of maximum stimulator output shows a further trend towards a larger MEP amplitude and MEP area. It was not possible to win all AWS that participated in the ICI/ICF session to participate in the MEP input–output curve session. Thus, the data result from a subsample of AWS of the first experiment which limits their interpretation.

A limitation of the study is the relatively small number of recorded responses in the paired-pulse condition, eight MEPs per conditioning-test interval. This possibly does not account for the variability of the MEP-peak-to-peak amplitude. We had to limit the amount of trials due to the fact that the recordings from the tongue are not as comfortable as recordings from small hand muscles. Discomfort results from the simultaneous stimulation of the peripheral face muscles primarily the eye-muscles and the jaw; and the increased salivation triggered by the mouthpiece.

4.4. Implications

We specified a reduced and possibly delayed SICI in the right side, and increased ICF in the left side. As the final cortical processing stage for voluntary movements, the primary motor cortex is a critical site for the integration of movement selection, initiation and prevention processes. Upcoming studies might further elucidate state-dependent modulation of intracortical inhibition to reveal pathomechanisms of dysfluent speech production in stuttering. Furthermore, single-pulse and paired-pulse TMS can be employed to study underlying physiological mechanisms of a pharmacologically induced enhancement of speech fluency and to compliment current pharmacological approaches.

Disclosure

The authors report no conflicts of interest.

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